

INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Article 36 and Rule 70)

REC'D 12 APR 2005

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Applicant's or agent's file reference P1362PC	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/ES 03/00635	International filing date (day/month/year) 16.12.2003	Priority date (day/month/year) 18.12.2002
International Patent Classification (IPC) or both national classification and IPC G01N33/68		
Applicant ONE WAY LIVER GENOMICS, S.L. et al.		

- This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
- This REPORT consists of a total of 6 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

 These annexes consist of a total of sheets.

- This report contains indications relating to the following items:
 - I ☒ Basis of the opinion
 - II ☐ Priority
 - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☐ Lack of unity of invention
 - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - VI ☐ Certain documents cited
 - VII ☐ Certain defects in the international application
 - VIII ☐ Certain observations on the international application

Date of submission of the demand 12.07.2004	Date of completion of this report 11.04.2005
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**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/ES 03/00635

1. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-20 as originally filed

Claims, Numbers

1-14 as originally filed

Drawings, Sheets

1/5-5/5 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/ES 03/00635

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-14

because:

☒ the said international application, or the said claims Nos. 1-14 with respect to industrial applicability relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the Standard.

☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-14
	No: Claims	
Inventive step (IS)	Yes: Claims	1-14
	No: Claims	
Industrial applicability (IA)	Yes: Claims	
	No: Claims	1-14

2. Citations and explanations

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/ES 03/00635

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/ES 03/00635

Item III:

Claims 1-13 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT due to item a) of claim 1 ("obtaining a liver tissue sample from a subject"). Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Item V:

Reference is made to the following documents:

- D1: LU S.C. ET AL: 'Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation.' PNAS vol. 98, no. 10, 08 May 2001, pages 5560 - 5565, XP002980762
- D2: MATO J.M. ET AL: 'S-Adenosylmethionine: a control switch that regulates liver function.' FASEB JOURNAL vol. 16, 2002, pages 15 - 26, XP002980763
- D3: ZATLOUKAL K ET AL: 'Alcoholic and nonalcoholic steatohepatitis.' REV. ESP. PATOL. vol. 32, no. 3, 1999, pages 293 - 294

The subject-matter of the interfering document D4 "SANTAMARIA E. WT AL: 'Functional proteomics of nonalcoholic steatohepatitis: Mitochondrial proteins as targets of S-adenosylmethionine.' PNAS vol. 100, no. 6, 18 March 2003, pages 3065 - 3070" refers to relevant subject-matter.

The above document is published after the present application's priority date, but before its filing date and is therefore relevant for those parts of the present application, if any, which do not have a valid claim to priority.

The priority of the present application has not yet been checked.

1. Articles 33(2) and (3) PCT

1.1. The subject-matter of **independent claim 1 is novel**, as none of the available prior

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/ES 03/00635

art documents discloses a method for the diagnosis of NASH.

D1 reveals that nonalcoholic steatohepatitis is developed in mice missing MAT1A. Moreover, patients with mutations in MAT1A may be more susceptible to liver injury (p 5565, col 2, last para).
No diagnostic use of MAT1A is disclosed.

D3 describes a hallmark lesion in hepatocytes which is the appearance of cytokeratin 18 containing cytoplasmic inclusions, termed Mallory bodies. This is accompanied by a disruption of the cytokeratin intermediate filament cytoskeleton in NASH (p 293, col 2, para 1-2).
No diagnostic use of cytokeratin 18 is disclosed.

The subject-matter of **independent claim 1 is furthermore inventive** (Article 33(3) PCT), as none of the available prior art documents, either if taken alone or in any combination, reveals the claimed subject-matter.

The same is considered to apply to **dependent claims 2-13**.

- 1.2. The subject-matter of **independent claim 14 is novel and inventive** for analogous reasons as independent claim 1.

REPLACEMENT PAGE

11. A method according to claim 9, which comprises the detection and quantification of the levels of, at least, two proteins, each one independently selected from APA1, ATPB, LKHA, K1CR and PHB1.

12. A method according to claim 9, which comprises the detection and quantification of the levels of three or four proteins, each one independently selected from APA1, ATPB, LKHA, K1CR and PHB1.

13. A method according to claim 9, which comprises the detection and quantification of the levels of proteins APA1, ATPB, LKHA, K1CR and PHB1.

14. The use of a protein selected from apolipoprotein A1 (APA1), mitochondrial ATPase β subunit (ATPB), leukotriene A₄ hydrolase (LKHA), keratin 18 (K1CR), guanidinoacetate N-methyltransferase (GAMT), superoxide dismutase (SODC), albumin (ALBU), antioxidant protein 2 (AOP2) (isoforms 1 and 2), prohibitin 1 (PHB1), methionine adenosyl transferase (MAT), long-chain acyl-CoA dehydrogenase (ACDL), selenium binding protein (SBP), and their combinations, in an *in vitro* method to diagnose NASH or to evaluate the susceptibility of a subject to develop NASH.